Source	Sampling intensity	Core size	Cores collected/area	Sample size processed	Total soil collected
Netherlands Been&Schomaker 1996	Grid 5 x 5 m [5.5 x 5.5 yd]	52g [1.83 oz]	(~133cores) 0.33ha [0.82 acre]	not stated	6.9kg [15.2lb]
Netherlands statutory	Grid 7.5 x 7.5m [8.2 x 8.2 yd]	3.3g [0.12 oz]	(~61 cores) 0.33ha [0.82 acre]	not stated	200g [7.05oz]
EPPO (1998) (Southey, 1986)	Grid pattern	4-5 ml, 5cm deep [1.97 in]	100 cores (e.g. 4ha) [9.88 acres]	400ml (500g) [1.1lb]	400ml
Standard number of samples/ha in some countries			4 samples/ha [2.47 acre], 1 sample min for < 1/4 ha [0.61 acre]		
Pickup 2002 SEERAD based on EPPO	W-shaped path	(~7ml)	Excess of 70 cores/4ha [9.88 acres]	500ml	500ml
The Netherlands					6-11kg [13.27-24.25 lb]
Trudgill et al 2003 Current sampling strategies			4ha [9.88 acres]	100g [3.53oz]	
Minnis et al 2002	Grid	20cm by 2.5cm [7.87in by 0.98in]	50 cores/4ha [9.88 acres] (1core/800m^2) [0.2 acre]	200g [7.05oz]	2kg [4.41lb]

Source	Sampling intensity	Core size	Cores collected/area	Sample size processed	Total soil collected
Reid&Pickup 2005 SEERAD		5cm by 1cm [1.97 in by 0.39 in]	70-120 cores/4ha [9.88 acres]	500ml	500ml
M. Phillips 2006 TWG (system used for over 40 years)		5 cm by 1 cm	70-80 in wet sticky clay soil 70-120 or more in dry sandy clay soil 4ha [9.88 acres]	500ml	
EU Directive M. Phillips and J. Pickup 200g TWG (based on 1 live cyst to be found, any cyst in sample will be found, 90% detection)	Grid not less than 5m [5.5yd] in width and not more than 20m [22yd] in length between points		100 cores/ha [2.47 acre]	Standard size of 1500ml/ha [2.47 acre] (entire sample is processed)	
1st proposal (50cysts/kg, 0.3 million cysts/ha, 90% detection) May provide a suitable sampling rate for earlier detection of PCN in areas where PCN freedom is the expectation.			1ha [2.47 acre]	10 liters	

Revised Proposal (one single foci with a peak density of 100 cysts/kg [2.2lb]+ 3 smaller foci of 50 cysts/kg [2.2lb] within 1 ha [2.47 acre], 1.55 million cysts/ha [2.47 acre], 92% detection)			1 ha [2.47 acre]	1500ml	
EU PCN Directive Jon Pickup Email 2006 (peak density of 50 cysts per kg [2.2lb] in 1 ha [2.47 acre], 40% detection)	Grid of 6 x 16.7 m [6.56 x 18.26 yd]		100 cores/ha [2.47 acre]	1.5 liters/ha [2.47 acre] (peak density of 50 cysts/kg [2.2lb] in 1 ha [2.47 acre], 40% detection) (10 liters, 90% detection)	

Sampling Methods
Information below was taken directly from the referenced documents:

EPPO Reporting Service
1996 No. 10
and
Been, T.H., Schomaker, C.H. 1996. A new sampling method for the detection of low population densities of potato cyst nematodes (*Globodera pallida* and *G. rostochiensis*). Crop Protection, 15(4), 375-382.
"A new sampling method to detect low populations densities of *Globodera rostochiensis* and *G. pallida* (EPPO A2 quarantine pests) has been developed in the Netherlands. With this method it is possible to detect small infestations with a predefined probability, and therefore to take better decisions on the nature and extent of control measures, as the final aim is to reduce the use of nematicides.
Analysis of intensive sampling data from 40 patchy infestations representing all growing areas in the Netherlands provided a model of distribution (simple exponential model) describing expected cyst numbers within infestations. The authors then defined a standard focus with a central population density of 50 cysts/kg of soil which should be detected with a probability of 90 %, and used a special computer programme to simulate sampling procedures in order to define an optimal sampling grid. As a result, grid dimensions near 5 x 5 m with a core size of 52 g (sample size 6.9 kg/0.33 ha) were recommended as being the best compromise between the two following conflicting aims: to minimize sample size and variance of detection probability, and to minimize time needed to collect and process the samples. When compared with the statutory soil sampling procedure still used in the Netherlands of 7.5 x 7.5 m grid and core size of 3.3 g (sample size of 200 g/0.33 ha), and with an average detection probability of 90 %, the new sampling method detects foci with central densities a hundred times smaller, and costs only 3-4 times as much to perform. The new method can detect foci with a central density of 150 cysts/kg soil and higher with high probability (approx. 100%). If an infestation focus is present in a field, but not detected with the new sampling method, it can be very small. The authors noted that practical problems created by a more intensive sampling procedure can be solved by using automated sampling devices mounted on a jeep and elutriators for the extraction of cysts from soil samples of up to 3 kg.

The authors concluded that with this new sampling procedure, it has been possible to reduce very significantly (80 to 90%) the use of soil fumigants in seed and ware potato-growing areas in the Netherlands.

The wider the (sampling) grid, the larger the soil sample needed to detect the standard focus with a 90% probability, although the relationship is by no means linear. For instance, the amount of soil required to detect the standard focus with an average detection probability of 90% is 6.7kg when using a 2.5 x 2.5 m grid and 15.6kg using an 11 x 12 m grid. Further, an increase in grid size in the direction of cultivation proved to be less sensitive than the same increase perpendicular to that direction.
As the new methods require the collection of large soil samples, the area from which a sample is taken was reduced from 1/3 ha to 1/9 ha."

EPPO Standards
Phytosanitary Procedures
Globodera pallida* and *G. rostochiensis
Soil Sampling Methods 1998

"The basic requirements of soil sampling to detect or estimate potato cyst nematodes in the soil are that: (1) the final sample examined in the laboratory is large enough to achieve the required accuracy and/or sensitivity; (2) the sample is derived from sufficient points to ensure that it is representative of the area sampled, i.e. as far as possible heterogeneity (patchiness) in the nematode distribution is overcome; (3) the laboratory processing procedures are as efficient and free from operator error as possible, so as to give accurate and consistent results. The problems of sampling errors in this work were reviewed by Southey (1974) and discussed in more detail by Barker & Campbell (1981) and Seinhorst (1982). EPPO's earlier recommendations on soil sampling (OEPP/EPPO, 1955) are replaced by the present quarantine procedure.

A suitable sampling procedure (Southey, 1986) is to take 100 cores (borings) of 4-5 ml of soil with a half-cylindrical sampling tool, from not deeper than 5 cm in the soil. These sub-samples are distributed on a grid pattern throughout the plot and collected in a polyethylene bag to provide a sample of 400 ml (500 g) which is processed completely in the laboratory.
Plant protection services in different countries adopt different standards for the size of sampled area. In some countries, one sample is taken from each area for which a separate decision on presence/absence of cysts is to be made, irrespective of the size of the area, but up to a maximum of the usual field size in that country (e.g. 4 ha). In this case, the probability of detecting a given infestation level will be the same whatever the area sampled. In other countries a standard number of samples per ha is required (e.g. 4 samples per ha, 1 sample being the minimum requirement for areas less than ¼ ha). This method ensures consistency by the inspectors in sample-taking. It should be noted, however, that from a statistical point of view larger areas are sampled more intensively

by this procedure than smaller areas and therefore smaller areas are less likely to be rejected because of the detection of cysts."

Haydock, P.P.J. & Perry, J.N. (1998). The principles and practice of sampling for the detection of potato cyst nematodes. pp. 61-74 in: *Potato Cyst Nematodes: Biology, Distribution and Control.* (eds. R.J. Marks & B.B. Brodie). CAB International, Wallingford.

"The European and Mediterranean Plant Protection Organization (EPPO) recognizes two methods for sampling potato cyst nematodes (PCN), *Globodera rostochiensis* (Woll.) and *Globodera pallida* Stone: (i) taking soil samples and extracting the cysts in a laboratory; or (ii) the removal of growing plants and the examination of their roots for developing females (Anon., 1991).

Whether for detection or the estimation of PCN population densities, the basic requirements of sampling soil are the same.

1. The final sample examined in the laboratory must be large enough to achieve accuracy and/or sensitivity.
2. The sample must be derived from sufficient points to ensure that it is representative of the area sampled, i.e. that, as far as possible, any heterogeneity in the nematode distribution is overcome.
3. The laboratory processing procedures must be as efficient and free from operator error as possible, so as to give accurate and consistent results (Anon., 1991).

Foci are often elliptical in shape, with the largest population density in the centre of the patch.

(Schomaker and Been, 1992) They denoted a scheme in which a sample is taken every *a* metres in one direction and every *b* metres in a direction perpendicular to this as an '*a* x *b* m sampling grid'; noting that the density of sampling from such a grid is one sample per *ab* m^2. To detect a focus of 50 cysts kg^{-1} soil with a probability of 90% in a 1/3 ha field, a sample size of 6 kg soil would be needed for a 6 x 4 m sampling grid (24 m^2), but this would be increased to 11kg for a 4 x 12 m grid (48 m^2).

Been and Schomaker (1996) developed this work further to publish a new sampling method for PCN for use in the Netherlands based on a resolution of the two conflicting aims of: (i) minimizing the variance of the detection probability and the sample size; and (ii) minimizing the time needed to collect and process the soil samples. They recommended sampling on a 5x5 grid, whereby a focus of PCN infestation with a maximum density of 150 cysts kg^{-1} soil (and higher) would be detected with a near 100% probability.

Turner (1993) investigated the variation in population detection success for statutory sampling. For 42 fields of 1-2 ha each, taking 36 cores per field, a positive linear relationship was reported between the number of times a field was sampled and detection success ($r = 0.992$). For each additional sample, the PCN detection rate was increased by 7.85%. Increasing the number of sampling points above 50 cores per sampled area (while retaining an equal-sized total sample) did not significantly increase detection rates. When a PCN population has reached levels where it is detectable with a realistically sized soil sample (approximately 0.5 kg), it is already widely distributed throughout the field.

Auger types

…the Dutch system (to avoid subsampling and the introduction of additional error), an 8 cm x 1 cm auger is often used. Turner (1993) compared augers of 3, 10 and 12 ml volume but found no significant difference in detection efficiency between the three augers when the number of probes was adjusted to give a standard 360ml sample.

Sampling patterns

(based on 45 fields sampled 6 times each by 4 different methods, Turner 1993)
…no significant differences in PCN detection were found between different sampling methods. To minimize the costs of sampling, the perimeter method is the most efficient; it takes less time because shorter distances are walked. In practice, this method is avoided in favour of zigzag sampling, largely because of the perceived dangers of headland and field entrance contamination. Operators also, intuitively, prefer to cover the sampling area.

Perry (1996) analyzed the data of Turner (1993) using spatial analysis by distance indices (SADIE) (Perry, 1995). Perry concluded that the sampling pattern may indeed be an important determinant of the success in detecting nematode infestations and that the zigzag plan should be adopted in preference to the perimeter plan, as it represents as much of the field as possible, and increases the chance of detection infestations over a range of levels of aggregation (Perry, 1996).

Recommended sampling strategies

Soil sampling systems differ between countries and their use depends on whether statutory testing or population density estimation is the aim.

For routine estimation of PCN, Southey (1986) recommended taking 50-60 (minimum 25) core samples distributed systematically, for example following a W-shaped path, over a maximum area of 4 ha.

Church et al. (1959) recommended that a total of 2.5 kg (minimum 1 kg) be taken and then subsampled before laboratory extraction.

If certification is the objective, the maximum chance of detection should be the aim, but using a test of equal stringency for all crops subject to the same type of regulation. It is recommended by EPPO that 100 cores (each of 4.5 ml volume) are taken, using a cheese-type corer to a maximum depth of 5 cm. The cores are taken on a systematic grid pattern over the whole of the area and then the total sample of approximately 400 ml is processed in the laboratory.

Since 1966 in Scotland, the Department of Agriculture and Fisheries for Scotland (DAFS) (now Scottish Office: Agriculture and Fisheries Department; SOAFD) have used the modified Dutch method, which aims to take approximately 500 ml of soil in cores 5 cm x 1 cm. The number of cores taken varies from 70 to 120 depending on soil conditions. As the objective is detection of PCN, field operators are advised to take cores on a W-shaped pattern over the sampling area, concentrating on obvious danger spots, e.g. gateways and boundaries of neighboring suspect fields (Pickup, 1996).

The maximum chance of detection requires sufficient cores to be taken to account for heterogeneity of distribution, followed by the processing of as large an amount of soil as possible. In practice, c. 500 g of soil is usually the largest amount that can be processed. The ideal might, therefore, be to take 50 or more cores of 8 ml each, giving 400 ml or 500 g of soil, so that the whole sample can be processed and subsampling errors avoided (Haydock and Evans, 1994).

Table 4.2 Examples of sampling soil to detect PCN for statutory purposes.

Country	Area to provide one bulk sample (ha)	Pattern of cores	Number of cores	Core size	Total bulked sample	Source
USA	0.2 max.	Grid	56		1.8-2.7 kg	Anon. (1992)
The Netherlands	0.33 max.	7.5 m x 7.5 grid	c. 60	3.33g	200ml	T. Been, IPO-DLO,Wageningen, 1997, pers. comm.
Bolivia	0.5-2.0	Grid	50 ha^{-1}	2.5cm x 20cm	4kg	J. Franco, PROINDA, Cochabamba, 1996, pers. comm.

Northern Ireland	2.0 max	Grid	30-36	8.5cm x 2.0cm	360g	Turner (1993)
England	4.0 max	Grid or W-shaped	55-60	5.0cm x 1.0cm	500ml	B. Ellam, MAFF York, 1996, pers. comm..
Scotland	4.0 max	W-shaped	70-120	5.0cm x 1.0cm.	500ml	Pickup (1996)
South Africa	4.5 (avg)	Grid	144 ha^{-1}	50 ml		K.P.N. Klenynhans, ARC-PPI, Pretoria, 1996, pers. comm..

Table 4.3 Examples of sampling soil to estimate PCN population density for commercial advisory purposes.

Country	Area to provide one bulk sample (ha)	Pattern of cores	Number of cores	Core size	Total bulked sample	Source
The Netherlands	0.33 max	2.5m x 11.0m grid	120	5.0ml	600ml	T. Been, IPO-DLO,Wageningen, 1997, pers. comm
UK	1.0	W-shaped	32	2.5cm x 20cm	1570ml	A. Wade, Profarma, Shropshire, 1997, pers. comm
UK	4.0 max	W-shaped	50-60	2.5cm x 20cm	2kg	Southey (1996)
UK	4.0 max	W-shaped	40	2.5cm x 20cm	2kg	W. Lankford, Rhone-Poulenc, Ongar, 1997, pers. comm.
UK	4.0 max	W-shaped	40 (min)	2.5cm x 20cm	1kg (min)	M. Lole, ADAS, Wolverhampton, 1997, pers. comm.

California beet cyst nematode sampler
The California beet cyst nematode sampler was designed to be attached to the rear of an all-terrain motor tricycle. The mechanical sampler enables relocatable transects across fields to be established and sampling time to be reduced by 67% compared to sampling by hand. It is well suited for sampling cultivated soil for cyst nematodes (Cooke et al., 1979; Roberts and Thomason, 1981)."

Potato Cyst Nematodes – a technical overview for Scotland
Adapted by Dr. Jon Pickup, from overview for England & Wales by Dr. Sue Hockland, CSL, Sand Hutton, York. August 2002.
"Detection
Soil sampling is necessary to determine the presence or absence of PCN and thus determine which fields can be used for seed potato production. The sampling and extraction systems used by the Scottish Executive Environment and Rural Affairs Department (SEERAD) (based on the European and Mediterranean Plant Protection Organisation [EPPO] Quarantine Procedure for *G. pallida* and *G. rostochiensis*) provide a practical and reliable indicator of the presence of PCN.
Soil samples are taken from an area up to 4 hectares, usually by following a 'w'- shaped path and taking regular samples *en route* with a corer. Usually in excess of 70 cores are required to collect a 500ml sample for testing. The combination of producing seed potatoes only on land that has been officially tested and found 'free' from PCN, together with a tight tolerance for the quantity of soil permitted with seed tubers, has greatly reduced the risk of spread through seed potatoes.
Soil sampling methods promoted in The Netherlands use larger quantities of soil (6-11 kg of soil), and are often advocated in order to achieve higher levels of statistical confidence, but they are more costly. The EPPO sampling method is currently under review."

Trudgill, D.L., Elliott, M.J., Evans, K., Phillips, M.S. 2003. The white potato cyst nematode (*Globodera pallida*) – a critical analysis of the threat in Britain. Annals of Applied Biology, 143, 73-80.
"Both species of PCN are introduced pathogens dependent for their spread on the movement of infested planting material and of soil moved by machinery, wind, water and animals. Introductions are typically patchily distributed and, because they comprise few cysts, cause no damage and are almost impossible to detect (Been & Schomaker, 2000).

But, with current sampling strategies that typically involve processing only 100 g soil from 4 ha, it is only after several potato crops, when populations have become very large and widely distributed, that detection becomes likely (Trudgill *et al*., 2001). This is demonstrated by a simple calculation of the PCN population density that might be

represented by one cyst in a 200 g soil sample, as used by Minnis *et al.* (2002). Minnis *et al.* (2002) took 50 cores of soil from 4 ha (equivalent to one core 800 m-2) to give a total sample of *c.* 2 kg of soil. However, cysts were extracted from a mixed 200 g sub-sample (one tenth). Assuming a soil bulk density of 1.25 and a 25 cm plough depth, it can be calculated that one cyst (the minimum that can be detected) in such a sub-sample is equivalent to a population density of *c.* 62 million viable cysts in one 800 m2 block. To generate such a population would require five to seven potato crops, assuming that initially 10 cysts of wPCN were introduced and there was a 10- to 20-fold increase in cyst numbers with each potato crop. On a 1 in 5 rotation, this would take between 25 and 35 yr. Consequently, many recently infested fields will still be below the threshold for detection, especially as this simple analysis ignores complicating factors such as a patchy distribution (see Been & Schomaker (2000) for a detailed analysis)."

Reid, A., Pickup, J. 2005. Molecular characterization of a morphologically unusual potato cyst nematode. OEPP/EPPO Bulletin 35, 69-72.
"Agricultural staff of the Scottish Executive Environment and Rural Affairs Department (SEERAD) draw over 6000 pre-crop soil samples annually from
land intended for seed-potato production. These samples comprise approximately 500 mL soil and are made up from 70 to 120 cores of 5 cm length by 1 cm diameter, collected from an area of up to 4 ha."

From M. Phillips presentation at the TWG Boise meeting:
Statutory sampling in Scotland

- Approximately 500 ml soil, in cores of 5 cm x 1 cm, is collected from each sample unit 4 hectares.
- 70 to 80 in a wet, sticky, clay soil to 120 or more in a dry sandy clay soil.
- Sampling is biased towards detection, with greater concern over establishing presence or absence, than in assessing population levels.
- However, for over a forty years the system has proved practical in Scottish conditions

M. Phillips and John Pickup presentation:

- With reference to the sampling and testing for the official investigation… :
  sampling shall involve a sample with the standard size of at least 1500 ml soil/ha collected from at least 100 cores/ha preferably in a rectangular grid of not less than 5 meters in width and not more than 20 meters in length between sampling points covering the entire field. The whole sample shall be used for further examination.

How was this rate arrived at?

- Drawn up by Münster working group in May 2004
- Based on best available models of aggregated field distributions of PCN (Been & Schomaker - NL)
- Assumptions include:
- One live cyst to be found

- Any cyst present in the sample will be found
- Probability of detection should be at least 90%
- Increasing number of cores above 100/ha has relatively limited impact on probability of detection
- i.e. sampling rate comparable with EU Directives on quarantine bacterial pathogens: Ring Rot & Brown Rot

- 1st Proposal: aim of detection of a single focus of infestation within 1 ha with a peak density of 50 cysts/kg (0.3 million cysts/ha)
- Model - 90% detection chance requires 10 litres soil/ha
- Conclusion – Unrealistic sampling rate for routine testing within EU, therefore 90% chance of detection aimed at higher PCN populations

However, 10 litres soil/ha may provide a suitable sampling rate for earlier detection of PCN infestation in areas where PCN freedom is the expectation

- Revised Proposal: aim of detection of a single focus with a peak density of 100 cysts/kg and three smaller foci of 50 cysts/kg within 1 ha (1.55 million cysts/ha)
- Model - 1500ml sample - 92% probability of detection

Such a high level of PCN infestation is likely to have developed over several rotations of potato production

Emails to TWG from:
Dr. Jon Pickup, Scottish Agricultural Science Agency 5/05/06
"Many thanks for your invitation to join you in Idaho next week. Unfortunately I already have commitments here in Scotland. However, having looked at the correspondence that you have sent out, I do have a few thoughts to offer you.

I suppose the greatest concern for you is that as you are detecting PCN in field soils, it is most likely that the contamination was introduced into the land with contaminated seed potatoes going back several rotations. Precisely how long ago will depend on the frequency of potato production, the suitability of the varieties to PCN multiplication (most likely all have been equally susceptible) and the environmental suitability (especially soil type). This leads on to an urgent need to establish the risks of spread of PCN from this production area since the original introduction. Following on, you would probably wish to trace the likely source of the introduction, especially if the seed potatoes planted in this area have all been produced locally.

As PCN infestation levels are likely to be low in many of the areas you will need to investigate, you will probably need to use the highest sampling intensity that resources will permit and I certainly wouldn't advocate sub-sampling, except as a means of not wasting effort when a smaller sample would be likely to reveal a positive result.

When sampling rates were looked at as part of the lead up to the proposal for a new EU PCN Directive, we relied extensively on the PCN distribution models developed by Thomas Been and Corrie Schomaker in the Netherlands and they used their SAMPLE V programme to look at the detection efficiencies of various sampling protocols. As a practical solution for routine testing of land prior to seed production, it was proposed that one core should be taken every $100m^2$ ideally on a grid of 6 by 16.7m (the latter being in the direction of cultivation), i.e. 100 cores per ha. Taking cores more frequently did not provide much greater confidence in detection, whereas taking more soil did. To obtain a 90% chance of detecting a single focus of PCN infestation with a peak density of 50 cysts per kg within a 1ha unit, a sample size of 10 litres would be required. The final conclusion within our working group was that a sample size of 1.5 l/ha was a more practical solution for routine testing, giving only a probability of 40% of detecting a single focus of a similar size. In cases where early detection of infestation has higher priority, such as when first infestations in an area have been found - as in your case, there are greater benefits to be gained from more intensive sampling."